1974

The Effects of Aging on Epithelial Tissue

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THE EFFECTS OF AGING ON EPITHELIAL TISSUE

by

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A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

JUNE

1974

TABLE III

Concentrations of Serum Proteins

Serum (mg per 100ml)

	IgG	IgA	IgM	Albumin	C'3 Complement
Inflamed group	1033	355	102	3040	211.6
Non-Inflamed group	988	192	167	3760	129.3

Pulp (mg per 100ml)

	IgG	IgA	IgM	Albumin	C'3 Complement
Inflamed group	97.2	7.75	0	96	26
Non-Inflamed	47	4	0	111.3	23

Normal Human Serum (mg per 100ml)[3]

IgG	IgA	IgM	Albumin	C'3 Complement
1200±319	288±121	145±100	4830±900	145±22

[3]Values supplied by Hyland Laboratories, Round Lake, Illinois variability of ±10% as per Fahey (1965).

LIFE

Leon A. Shepard was born in Trenton, New Jersey. He graduated from Trenton Central High School and attended Howard University in Washington, D.C. and graduated from Meharry Medical College, School of Dentistry in Nashville, Tennessee receiving the Doctor of Dental Surgery degree.

He served as a General Dental Officer with the United States Navy and began graduate studies in the Department of Oral Biology at Loyola University, Chicago, Illinois in September, 1972.

His clinical training was done in the Department of Orthodontics at Loyola University.

DEDICATION

To my mother and father, who long ago showed me the way.

ACKNOWLEDGEMENTS

To the members of my board, I wish to express my sincere appreciation for their help and support.

To Doctor Patrick Toto, my thanks for his knowledgeable advice.

To Doctor B. Jaraslov, my gratitude for his incisive constructive criticism.

To Doctor Bernard Pawlowski, my appraciation for his helpful assistance and his basic humanitarianism.

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CHAPTER I

INTRODUCTION

Studies on the effect of aging on cell density and mitotic activity in humans indicated that both variables increased with age. This was demonstrated by Marwah, Weinmann, and Meyer (1960); Gargiulo, Wentz, and Orban (1961) and Soni and Silberkweitt (1965); and Marwah, Meyer, and Weinmann (1956) and Marwah, Weinmann, and Meyer (1960), among others. This study was undertaken to compare the effect of age on the cell density and labelling index in experimental animals with its effect on humans.

CHAPTER II

REVIEW OF THE LITERATURE

Minot (1908) was the first to describe the mitotic index, i.e., the number of cells which are undergoing mitosis at any given moment per one thousand cells. It has been developed into a useful tool in evaluating mitotic activity.

Various organs have been studied using the mitotic index; Rollason (1949) studying renal tubular epithelium of rats observed considerable mitotic activity at two weeks of age which diminished constantly with aging.

Sulkin (1949) found that mitotic figures were infrequent in normal kidney epithelium of ninety-day old rats; that is, adult rats. Walker (1955) studied cellular proliferation in the transitional epithelium of the bladder of mice and reported high mitosis levels at birth diminishing at two weeks of age and reaching a very low level in adulthood. McCreight and Sulkin (1959) further observed mitotic activity in renal epithelium to be negligible in senile rats as opposed to young mature rats. In addition, intervention via a unilateral nephrectomy served to stimulate mitotic activity in younger animals much more than in older animals. Von Sallmann and Grimes (1966) studying the lens epithelium in rats found a decrease in mitotic activity during the first year of life.

When the oral cavity is studied, contrasting points of view emerge. Marwah, Meyer, and Weinmann (1956) reported the first mitotic index of human oral mucous membrane when they studied the epithelium of attached gingiva and demonstrated increases in both mitotic index and cell density with age. The authors concluded that it seemed that slowing down of tissue maintenance did not seem to occur with age in the case of epithelium. Marwah, Weinmann, and Meyer (1960) reported that there was an increase in mitotic index with age in non-inflamed human gingiva and that there was a similar increase in cell density. In addition, both mitotic activity and cell density showed an even greater increase in inflamed specimens. Gargiulo, Wentz, and Orban (1961) reported an increase with age in the mitotic activity of human oral epithelium. They attributed this to a retarding of the mitotic process due to exposure of the epithelium to hydrogen peroxide; and that, therefore, this was not a true reflection of mitotic activity. Hayes, Silberkweitt, Soni, and Simpson (1963) demonstrated lower mitotic activity in a group of young subjects in which normal gingival epithelium was studied. With age, increased mitotic activity was noted accompanied by decreased cell density. It was speculated that this might be due to an increase in cell size or increased cell size of dividing cells, or both. Soni and Silberkweitt (1965) studying human gingival epithelium found that in males with normal gingival tissue, the mitotic index increased with age while cell density decreased.

In males with inflamed tissue, both mitotic index and cell density decreased with age. In females, cell density decreased with age while mitotic activity increased in both normal and inflamed tissue. Silberkweitt, Soni, Stricker, and Salamat (1967) demonstrated that under the influence of dilantin therapy, gingival mitotic activity decreased in males of all ages while younger females showed more activity than older females. Cell density decreased for treated males and females. The results of these and a few related studies are summarized in Table I.

Sharav and Massler (1967) studying basal and suprabasal cells in rats 2, 9, 19, and 27 months of age found that the labelled population decreased from 2 to 9 months when palatal and tongue epithelial tissue were studied. They further contended that from 9 to 27 months, the labelled cell population remained constant while there was a decrease in total cells. What the authors referred to as the synthesis index was most affected by total number of cells. Thus, the synthesis index rose from 9 to 27 as the total cell count fell; whereas, it decreased in the growing animal from 2 to 9 months as the cell count increased.

TABLE I

EPITHELIAL MITOTIC INDEX AND CELL DENSITY

HUMAN GINGIVA

AUTHOR & YEAR	SUBJECTS	AGE	AVG. MI.	AVG. CELL DENSITY
Marwah, Meyer & Weinmann (1956)	Normal Males	25-34	.98	$55/(100u)^2$
		50-78	1.56	$73/(100u)^2$
Marwah, Weinmann and Meyer (1960)	Normal Males	25-34	.55	$49/(100u)^2$
		50-79	.84	$58/(100u)^2$
Inflammation Increased MI by 1 ½ to 3 times.				
Gargiulo, Wentz and Orban (1961)	Normal Males	22-27	.79	$62/(100u)^2$
		48-63	1.69	$70/(100u)^2$
	Treated With 30% Hydrogen Peroxide	22-27	6.50	$58/(100u)^2$
		48-63	8.76	$65/(100u)^2$
Silberkweitt, Soni and Simpson (1963)	Inflamed Females	5-13	.514	21.52-72.00
	Inflamed Males	5-13	.575	$26.30-73.23/cm^2$
Hayes, (1964)	Normal Females	5-12	.401	$30.05-96.57/cm^2$
	Normal Males	5-12	.454	$38.05-91.39/cm^2$
Krajewski, (1964)	Normal Females	20-35	0.74	$54/(100u)^2$
Soni and Silberkweitt (1965)	Normal Females	14-24	.656	$35.7/cm^2$
	Normal Males	14-24	.669	$35.5/cm^2$
	Inflamed Females	14-24	.798	$35.7/cm^2$
	Inflamed Males	14-24	.875	$33.8/cm^2$

TABLE I (cont'd)

AUTHOR & YEAR	SUBJECTS	AGE	AVG. MI.	AVG. CELL DENSITY
Silberkweitt, Soni Stricker and Salamat (1967)	Normal Females	8-22	.492	48.1/cm^2
	Normal Males	8-22	.485	49.3/cm^2
	Females Treated With Dilantin	8-22	.550	31.6/cm^2

Finally, Bullough (1949) in a study of mouse epidermis found increased mitotic activity in older animals. In a far-seeing conclusion, he felt that this contradicted the common concept of aging according to which it was held that bodily processes slow down and regenerative capacities diminish with age. He hypothesized that if his findings were true for other epithelial tissues, it pointed to the possibility of the weakening of the mechanisms by which normal cellular proliferation was controlled in the older individual. As Bullough pointed out, this theory lended itself well as a correlative factor in the greater propensity for malignancies to occur in old age.

This hypothesis must be coupled with the more recent notion advanced by Ryan (1974); there are many variables effecting mitotic activity and cell density, and if age is to be singled out for scrutiny, all other variables must be adequately controlled if one is to get an accurate scientific appraisal of the true effects of aging.

Cameron (1972) addressed himself to this problem when he attempted to control the labelling procedure itself in his study. He injected tritiated thymidine intraperitoneally in male Swiss Albino Mice 3 to 20 months old three times per day, 15 mc in 0.1 ml. H_2O. The animals were killed at 1 hour, 1, 2, 3, and 4 day intervals. Strips of tongue epithelial tissue 0.2 millimeter in length were sectioned and examined with a grid. There was an average 120-130 cells per 0.2 millimeters of tongue epithelium. There was no change in this number

from 3 months to 19 months. In general, there was a decrease in labelled cells from 3 months of age to 19 months of age. This coupled with the relatively constant cell population indicated that newly formed cells decrease with age. That is, the number of cells leaving the various compartments was less in old animals than in young animals, and the remaining cells lived longer (or were older in situ). This study and similar studies which preceded it are summarized in Table II.

TABLE II

AGE EFFECT UPON THE GENERATION ORAL EPITHELIUM

AUTHOR	ANIMAL	AGE	S	G_2	M	G_1	C
Reiskin (1967)	Hamster Pouch	Neo	6				26
		25 days	9-10				95
		155 days	9-10				155
Dhawan (1965)	Mouse Palate	60 days	8	.30	.7	96	105+
	Mouse Tongue Dorsum	60 days	8	.30	.7	96	105+
	Mouse Tongue Ventral	60 days	8.5	.30	.7	98	107+
Toto, Ojha (1961)	Mouse Tongue Ventral	60 days	8	.3	.7	96	104
Barakat, Toto (1969)	Mouse Palate	600 days	12	.15	.85	107	120+
Cameron (1972)	Mouse Tongue	3 mos.					107-
		13 mos.					122

THE VALUE OF TRITIATED THYMIDINE AS A LABELLING MATERIAL AND THE ADVENT OF AUTORADIOGRAPHY AS A SCIENTIFIC INVESTIGATIVE TOOL

In the matter of adequately controlling intervening variables, it become immediately apparent that a definitive method of determining when a cell was undergoing mitosis was of paramount importance. In the life cycle of a cell, one phase of the generation cycle was a duplication of the deoxribonucleic acid present in the nucleur. The fact that thymidine was incorporated into the DNA of proliferating cells had been demonstrated by Reichard and Estborn (1955) and Friedkin, Tilson, and Roberts (1956), and Friedkin and Wood (1956). The accomplishment of the synthesis of tritiated thymidine by Taylor, Wood and Hughes (1957) had made available thymidine labelled with a non-exchangeable long lived, low energy beta emitter (0.018 mev). Amano, Messier, and Leblond (1959) indicated that injection of tritiated thymidine produced radioactivity located solely in DNA. According to Rubini, Cronkite, Bond, and Fliedner (1959), tritiated thymidine emits beta rays with suitably low energy for recording on photographic emulsion. Thus, Toto and Ojha (1961) concluded that tritiated thymidine conveniently labels those cells in DNA synthesis and imports radioactivity high enough to permit the entry of adequate amounts of radioactive material to ensure production of autoradiograms of high resolution in a reasonable period of time and with minimal chromosomal injury.

As outlined by Borg (1967), autoradiography involved the placement of a photographic emulsion in contact with a slide containing a radioactive substance. A latent image was formed on the film and was made evident by photographic developer. Silver grains were then apparent lying above the specimen. Jensen and Toto (1968) had utilized tritiated thymidine and autoradiographic techniques to report the reduction of the percentage of labelled cells with age in the periodontal ligament of the maxillary first molar of the rat. Toto and Borg (1967) employed similar techniques and reported similar effects of age in the periodontium of mice. Pinzon, Toto, and O'Malley (1965) similarly showed a reduction in labelling index in the study of aging rat pulp.

Thus, the established validity of tritiated thymidine as a labelling material and the established validity of autoradiography as an observational tool lend themselves to the task at hand, i.e., an observation of the effects of aging on human oral epithelial tissues.

CHAPTER III

METHODS AND MATERIALS

Thirty Simonson strain rats, purchased from Locke Ericson Breeders, Forest Park, Illinois, were used in this study. The animals were received in good condition from the Breeders and were maintained on standard laboratory chow* and water ad libitum. They were divided into three groups of ten animals each according to age. Group 1 consisted of one-month old rats; Group 2 of 10-month old rats; and Group 3 of 15-month old rats. All groups were considered adult animals. The animals were injected intraperitoneally with tritiated thymidine acquired from Schwarz Bioresearch, Orangeburg, New Jersey (the specific activity was 1.9 curies per millimole) at a dose rate level of one microcurie per gram of body weight.

One hour after injection, the animals were sacrificed by an overdose of ether.

The area selected for observation was the tongue. The tongues were removed from the mouths and cut mid-sagitally.

The tissues were fixed in 10% neutral formalin for 24 hours. They were washed in running water, dehydrated with ascending ethyl alcohols, cleared in xylene and embedded in paraffin. Eight sections of five microns thickness were made from each block of tissue. Autoradiograms were prepared using Kodak Nuclear Track Emulsion Type NT B3. These sections were exposed in light tight slide boxes kept in

the freezer at 40C for one week. They were subsequently stained with nuclear fast red and indigo carmine.

The cell density in the epithelium of the ventral surface of the tongue was calculated by counting the cells in three groups of rats using a reticule 100 microns square covering a field 110 microns square at a magnification of 450 times. Using the basement membrane as a guide, the basal cells were always included.

Thirty random reticules were counted per specimen, and the total of labelled and unlabelled cells was recorded. Before a cell was considered as being labelled, it had to meet the following criteria:

1. The grains had to be clustered over a nucleus.
2. There had to be three grains or more per cell above background radiation.

The labelling index was calculated as the number of labelled cells per 1,000 cells.

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*Acquired from Ralston-Purina Company.

CHAPTER IV

FINDINGS

A. CELL DENSITY

The cell density (the number of cells per 110 square microns at 450 magnifications) was determined for each specimen. The mean cell density for the 30-day old group was 247.06. The mean cell density for the 10-month specimens was 240.03. The mean cell density for the 15-month specimens was 263.62. (Tables III, IV, and V). Statistically, there were no significant differences in densities between groups:

30-Day Old Specimens

The range of number of cells per 110 square microns at 450 magnifications was 108.97 to 320.33. The standard deviation was 75.69. The total number of cells seen per 110 square microns at 450 magnifications for all specimens was 2470.64. These findings are summarized in Table III.

10-Month Old Specimens

The range of cell densities was 124.83 to 330.40. The standard deviation was 55.90. The total number of cells seen per 110 square microns at 450 magnifications for all specimens was 2400.26. These findings are summarized in Table IV.

15-Month Old Specimens

The range of cell densities was 157.59 to 391.27. The standard deviation was 80.29. The total number of cells seen per 110 square

These findings are summarized in Table V.

In comparing the 30-day old group to the 10-month old group, the t value was .6650. The t value comparing the 30-day old group to the 15-month old group was -.132. The t value for the comparison of the 10-month old group to the 15-month old group was -.788. In all cases, the value of t at the 05 confidence level was $\pm$ 2.10 and the 01 confidence level was $\pm$ 2.88 given 18 degrees of freedom. These findings are summarized in Table VI.

B. LABELLING INDEX

The labelling index (the number of cells labelled with tritiated thymidine per 1000 cells) was determined for each specimen in each group. The mean labelling index for the 30-day old group was 46. The mean labelling index for the 10-month old group was 31.83. The mean labelling index for the 15-month old group was 74.

30-Day Old Specimens

The range of the number of labelled cells per 1000 cells was 23 to 83. The standard deviation was 16.89. The total number of labelled cells seen was 460. These findings are summarized in Table III.

10-Month Old Specimens

The range of the number of labelled cells per 1000 cells was 0 to 90. The standard deviation was 28.69. The total number of

labelled cells seen was 318.16. These findings are summarized in Table IV.

15-Month Old Specimens

The range of the number of labelled cells per 1000 cells was 45 to 131. The standard deviation was 23.58. The total number of labelled cells seen was 740. These findings are summarized in Table V.

In comparing the 30-day old group to the 10-month old group, the t value was 1.35. The t value comparing the 30-day old group to the 15-month old group was -3.05. The t value for the comparison of the 10-month old group to the 15-month old group was -3.59. In all cases, the value of t at the 05 confidence level was $\pm$ 2.10; and at the 01 confidence level, it was $\pm$ 2.88 given 18 degrees of freedom. Thus, the Null hypothesis was rejected in the first instance $\bar{x}_{30}$ is statistically equal to $\bar{x}_{10}$ months. However, the Null hypothesis is accepted in the latter two cases $\bar{x}_{30}$ days = $\bar{x}_{15}$ months and $\bar{x}_{10}$ months = $\bar{x}_{15}$ months. These findings are summarized in Table VI.

TABLE III

30 DAY SPECIMEN	RETICULES COUNTED	NUMBER OF CELLS COUNTED	NUMBER OF CELLS LABELLED	TOTAL NUMBER OF CELLS	NUMBER OF CELLS PER RETICULE	NUMBER OF LABELLED CELLS PER 1000 CELLS
#1	29	2897	263	3160	108.97	83
#2	30	3525	180	3705	123.50	49
#3	30	7160	460	7620	254.00	60
#4	30	6356	252	6608	220.27	38
#5	30	8520	350	8870	295.67	39
#6	30	8146	429	8575	285.83	50
#7	30	8535	459	8994	299.80	51
#8	30	7275	246	7521	250.70	33
#9	30	9029	318	9347	311.57	34
#10	30	9391	219	9610	320.33	23
TOTAL	299	70,834	3176	74,010	2470.64	460
					MEAN & STANDARD DEVIATION	MEAN & STANDARD DEVIATION
					$\bar{x}$= 247.06 s = 75.69	$\bar{x}$ = 46 s = 16.89

TABLE IV

10 MONTH SPECIMEN	RETICULES COUNTED	NUMBER OF CELLS COUNTED	NUMBER OF CELLS LABELLED	TOTAL NUMBER OF CELLS	NUMBER OF CELLS PER RETICULE	NUMBER OF LABELLED CELLS PER 1000 CELLS
#1	30	3404	335	3739	124.83	90
#2	30	7360	582	7942	264.73	73
#3	30	6019	202	6221	207.37	32
#4	29	6754	240	6994	241.17	34
#5	30	6350	1	6351	211.70	.16
#6	30	6034	272	6306	210.20	43
#7	30	7650	315	7965	265.50	40
#8	30	9634	278	9912	330.40	28
#9	30	8686	0	8686	289.53	0
#10	30	7325	320	7645	254.83	42
TOTAL	299	66,516	2545	71.761	2400.26	318.16
					MEAN & STANDARD DEVIATION	MEAN & STANDARD DEVIATION
					$\bar{x} = 240.03$ $s = 55.90$	$\bar{x} = 31.83$ $s = 28.69$

TABLE V

15 MONTH SPECIMEN	RETICULES COUNTED	NUMBER OF CELLS COUNTED	NUMBER OF CELLS LABELLED	TOTAL NUMBER OF CELLS	NUMBER OF CELLS PER RETICULE	NUMBER OF LABELLED CELLS PER 1000 CELLS
#1	29	4237	333	4570	157.59	73
#2	30	5613	578	6191	206.37	93
#3	30	4576	687	5263	175.43	131
#4	29	9386	572	9958	343.38	57
#5	30	7539	500	8039	267.97	62
#6	30	11,077	861	11,738	391.27	73
#7	30	8921	700	9621	320.7	73
#8	30	9374	681	10,055	335.17	68
#9	29	5741	397	6138	211.66	65
#10	30	6491	309	6800	226.67	45
TOTAL	297	72,955	5618	78,373	2736.21	740
					MEAN & STANDARD DEVIATION	MEAN & STANDARD DEVIATION
					$\bar{x} = 263.62$ $s = 80.29$	$\bar{x} = 74$ $s = 23.58$

TABLE VI

STATISTICAL RESULTS

CELL DENSITIES

30-DAY OLD SPECIMENS	10-MONTH OLD SPECIMENS	15-MONTH OLD SPECIMENS
NUMBER OF CELLS / RETICULE	NUMBER OF CELLS / RETICULE	NUMBER OF CELLS / RETICULE
$\bar{x} = 247.06$	$\bar{x} = 240.03$	$\bar{x} = 263.62$
$s = 75.69$	$s = 55.90$	$s = 80.29$

+ TESTS

30 DAYS vs 10 MONTHS	30 DAYS vs 15 MONTHS	10 MONTHS vs 15 MONTHS
h: $\bar{x}_{30} = \bar{x}_{10}$	h: $\bar{x}_{30} = \bar{x}_{15}$	h: $\bar{x}_{10} = \bar{x}_{15}$
$t = .6650$	$t = -.132$	$t = -.788$
$df = 18$	$df = 18$	$df = 18$
$to5 = \pm 2.10$	$to5 = \pm 2.10$	$to5 = \pm 2.10$
$to1 = \pm 2.88$	$to1 = \pm 2.88$	$to1 = \pm 2.88$
$\therefore \bar{x}_{30} = \bar{x}_{10}$	$\therefore \bar{x}_{30} = \bar{x}_{15}$	$\therefore \bar{x}_{10} = \bar{x}_{15}$
ACCEPT H	ACCEPT H	ACCEPT H

TABLE VII

STATISTICAL RESULTS

LABELLING INDEX

30-DAY OLD SPECIMENS	10-MONTH OLD SPECIMENS	15-MONTH OLD SPECIMENS
NUMBER OF LABELLED	NUMBER OF LABELLED	NUMBER OF LABELLED
CELLS 1000 Cells	CELLS 1000 Cells	CELLS 1000 Cells
$\bar{x} = 46$	$\bar{x} = 31.83$	$\bar{x} = 74$
$s = 16.89$	$s = 28.69$	$s = 23.58$
30 DAYS vs 10 MONTHS	**30 DAYS vs 15 MONTHS**	**10 MONTHS vs 15 MONTHS**
$h: \bar{x}_{30} = x_{10}$	$h: \bar{x}_{30} = x_{15}$	$h: \bar{x}_{10} = x_{15}$
$t = .135$	$t = -3.05$	$t = -3.59$
$df = 18$	$df = 18$	$df = 18$
$to5 = \pm 2.10$	$to5 = \pm 2.10$	$to5 = \pm 2.10$
$to1 = \pm 2.88$	$to1 = \pm 2.88$	$to1 = \pm 2.88$
$\therefore \bar{x}_{30} = \bar{x}_{10}$	$\therefore \bar{x}_{30} = \bar{x}_{15}$	$\therefore \bar{x}_{10} = \bar{x}_{15}$
ACCEPT H	REJECT H	REJECT H

activity was found to be markedly decreased by McKellar (1949) studying the rat liver, McLeight and Sulkin (1959) studying rat kidney, Walker (1958) studying the rat lacrimal gland, Pinzon (1965) studying the rat pulp, and Jensen (1968) studying the rat periodontal ligament. On the subject of cell density, most authors, including Ring (1960), Klingsberg (1960), and Shklar (1966), have observed a decrease in cell density with age.

The results of this study as previously stated indicated no change in cell density as a function of age and an increase in mitotic activity. There are a number of possible explanations for these apparent contradictions. First of all, the anatomical area selected for study, i.e., the ventral surface of the tongue, suggests a possible explanation. This is an area subject to a certain degree of stress during function and is thus an area where a great deal of sloughing occurs. Thus, there is a constant stimulus to produce new cells to replace cells that have been sloughed; and the cell density remains constant. A possible explanation for the increase in labelling index may be found in the nature of the labelling process. Cells duplicate their DNA content prior to mitosis. One of the essential precursors of DNA synthesis is the thymidine; thus, when cells actively synthesizing DNA are exposed to tritiated thymidine, they incorporate it and become radioactive themselves. This radioactivity becomes the basis for observing "labelled" cells and establishing a labelling index.

Cells undergoing mitotic division distribute their radioactivity equally between two labelled daughter cells as demonstrated by Beagrie and Skouguard (1962). Thus, after mitosis, a rise in the labelling index occurs. Carrying this reasoning one step further, the possibility exists that these cells are in a prolonged state of pre-mitosis; therefore, many cells may be seen in DNA synthesis, and this reflects a "psuedo" high labelling index.

In this particular study, it may well be that the oldest rats may just be entering into what could be considered old age as seems to be indicated by the rise in labelling indices. This would correspond to an increased mitotic index in human epithelium. Thus, it might well be that 15 months of age is a significant transition between young and old Simonson rats. The insignificant differences in cell density and labelling index serve as a verification.

The age associated change in the labelling index is related to the reports in human studies which show an increase in mitotic index. However, human cell density studies show an increase in the cell population, although there is a reduction in cell size. It is possible that in the Simonson rat the labelling index first rises with increasing age and later may be followed by an increased cell density. However, such a fact requires the study of older rats.

Thus, it would appear that with the use of the labelling index and cell density as parameters, quantitation of age associated change is possible in the oral mucosa.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The ventral surface of the tongue was studied to compare some changes which occur with age. Thirty Simonson strain of albino rats were divided into equal groups of 30 days old, 10 months old, and 15 months old. They were injected intraperitoneally with tritiated thymidine. The animals were sacrificed, and the tongues were removed from the mouth and cut mid-sagitally and prepared as autoradiograms.

The following may be concluded from this study:

1. The ventral surface of the tongue seems to be a good site for cell counts.
2. The ventral surface of the tongue seems to maintain a constant cell density from 0 to 15 months in Simonson rats; however, an increase in labelling index is noted.
3. The use of labelling index may well be recommended as a parameter in studying age associated changes in rats.
4. Rats 15 months of age may well be at a point wherein they are in transition between young and old and will, therefore yield conflicting results concerning labelling indices and cell densities.

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APPROVAL SHEET

The thesis submitted by Dr. Leon A. Shepard has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science.

DATE: May 15, 1974

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